Microenviromental factors controlling macrophage polarization in atherosclerosis

Oscar M. Pello
Molecular and Genetic Cardiovascular Pathophysiology Group, Epidemiology, Atherothrombosis and Imaging Department; Centro Nacional de Investigaciones Cardiovasculares (CNIC), Melchor Fernandez Almagro 3, 28029 Madrid (Spain)

Abstract

Macrophages are critical for the initiation and perpetuation of intravascular inflammation through their ability to produce an array of cytokines and chemokines, to generate reactive oxygen species, and to process and present antigens to CD4+ T cells. Macrophages constitute a heterogeneous population of cells that are distinctly activated by various microenvironmenal signals; however, the mechanisms contributing to the generation of distinct macrophage phenotypes in the context of atherosclerosis remain unclear. This review summarizes the well-characterized factors that govern macrophage polarization toward a specific phenotype and function. Understanding the microenvironmenal factors that control macrophage polarization and the precise roles of distinct macrophage subsets could provide the basis for novel treatment strategies aimed at limiting the progression of atherosclerosis.

Introduction

Atherosclerosis and associated cardiovascular disease (acute myocardial infarction and stroke) are the leading causes of death in developed countries, and it has been estimated that by 2020 these disorders will be the main healthcare and socio-economic problem world-wide, in part due to progressive societal aging. It is well-established that progression from early lesion to vulnerable plaque involves the participation of numerous cellular and molecular inflammatory components. The most prominent immune cells that invade lesions are monocyte-derived macrophages (representing up to 60% of atheroma plaque mass) and T-lymphocytes. Both cell types produce a wide array of soluble inflammatory mediators (cytokines, chemokines) that are critical for disease initiation and progression. For this reason, atherosclerosis is now regarded not simply as a lipid metabolism disorder, but also as a chronic inflammatory disease, and macrophages play a central role in the atherogenic process as modulators of both lipid metabolism and immune response [1,2].

Macrophages, the mature form of peripheral blood monocytes within tissues, are specialized phagocytic cells involved in multiple processes, both in homeostatic conditions and during the immune response induced by tissue damage or exposure to pathogen [3,4]. Macrophages acquire specialized phenotypes in response to signals from the local microenvironmen that polarize them toward a specific activation state. Activation with IFNγ, alone or in combination with pathogen-derived signals such as LPS, leads to classical-activated macrophages, also known as M1 cells, which participate in pro-inflammatory type 1 immune responses. Exposure to other immune modulators results in profoundly different phenotypes. These include ‘alternatively-activated’ macrophages induced by IL-4 or IL-13, which are associated with type 2 immune responses, and a spectrum of macrophage phenotypes related to anti-inflammatory, angiogenic, and tissue-repair properties, induced by stimuli including TGFβ, immune complexes, glucocorticoids, and IL-10 [4-6]. Tissue-infiltrating monocytes are simultaneously exposed to differentiating and activating factors. Macrophages generated after monocyte stimulation with GM-CSF (granulocyte-macrophage colony-stimulating factor) are considered pro-inflammatory M1 macrophages, whereas monocytes differentiated and activated with M-CSF (Macrophage colony-stimulating factor) acquire an anti-inflammatory phenotype and are denominated M2 macrophages. However, several authors consider that under homeostatic conditions, M-CSF differentiates monocytes into resident macrophages, which subsequently acquire the M1 or M2 phenotype in response to activation stimuli [7,8]. These two hypotheses might not be mutually exclusive: under steady-state conditions, monocytes infiltrate all tissues and differentiate into resident macrophages. Subsequently, in response to activation stimuli, these macrophages will acquire a specific differentiated phenotype. In addition, all immune responses involve recruitment of circulating blood monocytes, and these monocytes will be exposed simultaneously to differentiation and activation signals, resulting in their maturation into macrophages with a specific phenotype.

Roles of macrophages in atherosclerosis

Recent work suggests that different stages in the progression of atherosclerotic disease are associated with the presence of distinct macrophage subtypes [9,10].

The initial lesion: In both humans and experimental animals, hypercholesterolemia leads to the accumulation of plasma lipoproteins in the extracellular matrix of the vessel wall, where they undergo oxidation [11]. These deposits of oxidized lipoproteins, called fatty streaks, are very abundant in early atherosclerotic lesions, and are rich in lipid droplets, apoptotic cells and tissue debris. Endothelial cells neighbouring fatty streaks become dysfunctional, and it has been proposed that the prevalence of M2 macrophages in early atherosclerotic lesions is a reparative mechanism that limits disease progression in the initial stages. TGFβ released by M2 macrophages inhibits the recruitment of inflammatory cells, and this is associated with a significant atheroprotective effect [12]. M2 macrophages also have an immunosuppressive action through the release of IL-10, which inhibits the secretion of inflammatory cytokines such as IFNγ from other macrophages and from T-cells [13]. M2 macrophages are also atheroprotective through their capacity to reduce inflammation...
by clearing apoptotic cells and tissue debris, a process known as eryocytosis [14].

**Plaque growth:** Defective eryocytosis and the accumulation of inflammatory signals, including modified LDLs, correlates with atherosclerosis progression, possibly due to increased secondary necrosis and recruitment of inflammatory cells. The accumulation of M1 macrophages in the damaged vessel wall has been shown to contribute to plaque expansion [9]. In general, M1-produced pro-inflammatory markers are elevated in patients with unstable angina and myocardial infarction, with high levels predicting a poor outcome.

Factors released by M1 macrophages include cytokines IL-6, IL-7 and IL-8, the soluble CD40 ligand (CD40L), C-reactive protein (CRP) and pentraxin-3 [15, 16]. Atherosclerotic macrophages also secrete TNFα, which contributes to scavenger receptor downregulation [17]. In addition to activating other inflammatory cells, most of the inflammatory molecules produced by M1 macrophages contribute to the differentiation of newly-recruited monocytes to the M1 phenotype, increasing the inflammatory reaction through a feedback loop. M1 macrophages also release vasoactive molecules such as nitric oxide, endothelins, and eicosanoids [18]. Reactive oxygen species generated by M1 macrophages induce lipoprotein oxidation, with significant cytotoxic consequences [19].

**Pro-M1 Factors in Atherosclerosis**

**The macrophage-differentiating stimulus: GM-CSF**

Human macrophages can be obtained in vitro by culturing CD14+ peripheral blood monocytes in the presence of GM-CSF. This cytokine is considered a differentiating and activating molecule and is needed for the survival of monocytes in vitro. Macrophages obtained by culture of monocytes in the presence of GM-CSF have adherent ‘fried egg’ morphology and are characterized by considerable expression of the inflammatory cytokines IL-1β, IL-8, IL-6, and TNFα and high expression of IL-23 and IL-12 [20].

Under basal conditions, endothelial cells, smooth muscle cells and macrophages express little GM-CSF in vivo, but expression is increased upon exposure to pro-atherosclerotic stimuli such as inflammatory cytokines or oxLDL [17, 21]. However, GM-CSF+ macrophages (CD68+ CD14 according to Waldo et al.) seem to constitute only a minor fraction of atherosclerotic macrophages, and indeed GM-CSF is not always detected in human atherosclerotic lesions [22].

**Th1 cytokines: IFNγ**

IFNγ is the most important Th1 cytokine inducing the acquisition of an M1 macrophage phenotype. These macrophages are effectors in type I immune responses, pathogen killing and anti-tumor defense [6, 23].

IFNγ mRNA is highly expressed in atherosclerotic lesions and correlates with plaque progression [24]. IFNγ-stimulated macrophages have a pro-atherogenic phenotype characterized by production of the inflammatory cytokines IL-12, IL23 and IL-1β. Although IFNγ-activated human and murine atherosclerotic macrophages show reduced expression of CD36 and SR-A receptors, they contribute to abnormal cholesterol homeostasis by reducing cholesterol efflux, accumulating cholesterol esters and forming foam cells as a result of increased ACAT-1 activity and reduced expression of 27-hydroxylase and the ABCA1 cholesterol transporter [25].

**Inflammatory molecules: TNFα, CD40L and C-reactive protein (CRP)**

In addition to their primary role in inflammation, most inflammatory cytokines produced by M1 macrophages also help maintain the pro-inflammatory phenotype (of newly recruited monocytes). TNFα released by inflammatory atherosclerotic macrophages contribute to atherosclerotic inflammation [26], and TNFα production also modulates macrophage phenotype: macrophages stimulated with TNFα display an M1 phenotype by downregulating scavenger receptor expression and foam cell formation. Moreover, TNFα has been shown to induce stronger activation of NF-κB in the presence of IFNγ, and this NF-κB activation is accomplished via increased production of reactive oxygen species and induction of inducible nitric oxide synthase (iNOS) to produce nitric oxide (NO) [27].

CD40 ligand (CD40L) is a 39-kd transmembrane member of the TNF family with a well-established role in atherosclerosis. High levels of CD40L have been detected in patients suffering from hypercholesterolemia, unstable angina, or acute myocardial infarction [28]. Binding of CD40L to CD40 promotes pro-inflammatory cytokine production, reduces NO bioavailability, and induces overexpression of adhesion molecules, in turn promoting leukocyte recruitment and atheroma formation [29]. In a recent study, Verreck et al. showed that treatment of GM-CSF-derived macrophages with CD40L induced the expression of IL-18, IL-6 and TNFα, suggesting a role for CD40L in the maintenance of the pro-inflammatory macrophage phenotype in atherosclerosis [30].

C-reactive protein (CRP) is considered a reliable predictor of adverse cardiovascular events [31]. Moreover, administration of human CRP to rats induces endothelial dysfunction and activation of NADPH oxidase, NF-κB, matrix metalloproteinase-9, tissue factor activity, and release of the pro-inflammatory cytokines IL-1 and IL-6CRP (32). CRP has also recently been shown to promote the differentiation of human monocytes into M1 macrophages that release large amounts of the pro-inflammatory cytokines IL-12, IL-1β, IL-6, TNFα and MCP-1 while upregulating expression of CCR2 [33].

**Pro-M2 Factors in Atherosclerosis**

**The macrophage-differentiating stimulus: M-CSF**

Stimulation of human CD14+ peripheral blood monocytes with M-CSF promotes monocyte survival and their differentiation into M2-like phagocytic macrophages that produce high levels of IL-10 [20]. M-CSF-derived macrophages are adherent, with a stretched, spindle-like morphology, and are better able than GM-CSF-derived cells to form foam cells. A phenotype favoring foam cell formation is supported by genetic profiling of M-CSF-differentiated primary mouse macrophages [8]. M-CSF upregulates enzymes involved in cholesterol biosynthesis and downregulates ATP-binding cassette transporter G1 (ABCG1), which is involved in cholesterol efflux. Moreover, in human monocyte-derived macrophages, oxLDL accumulation is higher in M-CSF-differentiated macrophages than in GM-CSF-differentiated cells, and correlates with the upregulation of CD36 and SR-A, membrane proteins involved in the uptake of modified lipids [8, 22].

In mouse and human lesions, M-CSF is detected both in healthy arteries and atherosclerotic lesions, and in the latter is associated with macrophage and foam-cell content and correlates with plaque progression [8, 21, 22, 34]. These data are consistent with a scenario in which M-CSF and GM-CSF both contribute to macrophage heterogeneity observed in plaques. Since M-CSF is constitutively expressed, macrophages infiltrating early lesions are likely to differentiate toward an M2-like phenotype. As the plaque progresses, oxLDL and other inflammatory stimuli could potentially increase the production of both M-CSF and GM-CSF, making the balance between...
cytokines, increased debris scavenging, and a pro-healing functional
mediated mainly by their anti-inflammatory properties [45].

human atherosclerotic plaques [44]. Nonetheless most authorities
others were able to show IL-10 mRNA expression in a number of
[43].

These macrophages are less prone to apoptosis, and show increased
foam-cell formation by upregulating CD36 and SR-A.

high production of IL-10 and TGFβ [42]. In mouse macrophages, IL-
act as safeguards that control and dampen immune responses through

Anti-inflammatory molecules: IL-10 and TGFβ

II-10-primed macrophages are often called ‘regulatory’ or M2c, and
act as safeguards that control and dampen immune responses through
high production of IL-10 and TGFβ [42]. In mouse macrophages, II-
10 increases foam-cell formation by upregulating CD36 and SR-A.
These macrophages are less prone to apoptosis, and show increased
cholesterol efflux mediated via PPAR-γ-induced ABCA1 expression
[43].

Frostegard et al. did not detect II-10 in human lesions [21], while
others were able to show II-10 mRNA expression in a number of
human atherosclerotic plaques [44]. Nonetheless most authorities
agree that the atheroprotective role of II-10-primed macrophages is
mediated mainly by their anti-inflammatory properties [45].

Macrophones activated with TGFβ are also included in the M2c class
because they show the hallmark downregulation of pro-inflammatory
cytokines, increased debris scavenging, and a pro-healing functional
program [46]. Blocking antibodies against TGFβ or treatment with
soluble TGFβ receptor II accelerates atherosclerosis, associated with a
significant loss of collagen content [47].

Role of Apolipoprotein E and LDLS in Macrophage Polarization

Apolipoprotein E (ApoE) is a major protein component of very-
low-density lipoproteins (VLDL) and high-density lipoproteins
(HDL) and the ApoE knockout (ApoE/-) mouse is the most widely
used atherosclerosis model. Elimination of ApoE in this model causes
severe hypercholesterolemia leading to spontaneous development of
atherosclerosis [48,49].

ApoE was recently shown to induce characteristics typical of
alternative activation in mouse macrophages, reducing the steady-
state production of M1 cytokines IL-12 and macrophage inflammatory
protein-1α while increasing the production of the M2 cytokines II-1RA
and GM-CSF in a concentration-dependent manner. These findings
indicate that ApoE shifts the balance from a pro-inflammatory to an
anti-inflammatory cytokine profile [50].

Macrophones stimulated with immunocomplexes are called
M2b. Oxidized LDLS (OxLDLS) are major autoantigens that
form immunocomplexes with anti-oxLDL antibodies present in
atherosclerotic lesions. Macrophones can ingest these complexes
via Fc-γ receptors, leading to their activation. Kadal and colleagues
recently proposed the existence of a third polarized macrophage sub-
type, specifically associated with the oxLDL-rich microenvironment
of atherosclerosis. This sub-type, termed Mox, develops in response
to atherogenic phospholipids via expression of the redox-regulated
transcription factor Nrf2, and has a lower phagocytic and chemotactic
capacity than the conventional M1 and M2 macrophages [51].

Concluding Remarks

Macrophones are central to the initiation and progression of
atherosclerosis. Different macrophage sub-types have recently been
shown to be involved in different stages of the disease. The phenotype
and activity of each macrophage subtype depend on the profile of
factors present in the local microenvironment. Understanding how
each cytokine influences macrophage phenotype is crucial for the
development of anti-atherosclerosis therapies, and several current
studies are characterizing these processes. Although characterization
of the effects of individual cytokines is crucial, it must be remembered
that in a living organism cells are exposed to multiple cytokines
simultaneously. Future studies of the role of macrophages in
atherosclerosis must therefore examine the effect of combined stimuli
on the phenotype and function of these cells.

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